First Report of Root Rot Caused by *Pythium spiculum* Affecting Cork Oaks at Doñana Biological Reserve in Spain

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Cork oaks (*Quercus suber* L.) are key tree species at Doñana Biological Reserve (DBR), Huelva, Spain. Sampling was conducted on a total of 13 trees exhibiting symptoms of decline (foliar wilting and defoliation, branch dieback, and root necrosis). In 2008. *Phytophthora cinnamomi* was isolated from feeder roots of one tree and *Pythium spiculum* from two additional oaks. In 2011, both pathogens were isolated from six oaks, only *P. cinnamomi* from three oaks, and only*Py. spiculum* from one oak. This expansion was associated with high winter rainfall levels since 2009 that led to long periods of soil flooding. While *P. cinnamomi* is well known to cause a root disease on *Q. suber*(2), *P. spiculum* is a newly described species isolated from *Quercus*,*Vitis*, *Prunus*, *Castanea*, and *Celtis* species, but its pathogenicity was demonstrated only on *Q. ilex* (syn. *Q. rotundifolia*) (1). Pathogenicity tests were conducted on 4-year-old *Q. suber* plants. Inocula consisted of two isolates of *Py. spiculum* from DBR (DO8 and DO36 from *Q. suber*). For comparison with these, three isolates previously tested on*Q. ilex* (1) were included: two isolates of *Py. spiculum*, PA54 (from *Q. suber*) and PE156 (from *Q. ilex*); and one isolate of *P. cinnamomi*, PE90 (from *Q. ilex*). All these isolates came from the Andalucía region, stored at the oomycete collection of the University of Córdoba, and showed a 99 to 100% homology with their expected ITS sequences in GenBank (DQ196131 for *Py. spiculum* and AY943301 for *P. cinnamomi*). Inoculum was prepared by shaking and mixing propagule-bearing mycelium produced in carrot broth petri dishes (20°C, 4 weeks) in sterile water, to produce a concentration of 3×10^4oospores $\times$ ml^{-1} (*Py. spiculum*) or 3×10^4 chlamydospores $\times$ ml^{-1}(*P. cinnamomi*). One hundred milliliters of inoculum was applied to each root (1). There were 10 inoculated plants per isolate and 10 non-inoculated control plants. All plants were waterlogged 2 days per week to favor root infection and maintained in an acclimatised greenhouse (12–28°C). Three months later, the inoculated plants showed symptoms of root necrosis that resulted in foliar wilting followed occasionally by defoliation. Control plants did not develop foliar symptoms nor root necrosis. Root damage severity assessed on a 0 to 4 scale (3) exhibited significant differences ($P < 0.05$) in relation to the control plants for all the isolates tested, with isolate PE90 (*P. cinnamomi*) and isolates PA54, DO8, and DO36 (*P. spiculum*) all averaging a root necrosis value of 2.5. Isolate PE156 of *P. spiculum*produced values of root necrosis (1.6 in average) significantly lower ($P< 0.05$) than the rest. This isolate belongs to the low virulence group of*P. spiculum* described on *Q. ilex* (1). The inoculated oomycete was always reisolated from necrotic roots and never from roots of control plants. To the best of our knowledge, this is the first report of *P. spiculum* as the cause of root rot of *Q. suber*.

References: (1) Romero et al. J. Phytopathol. 155:289, 2007. (2) Sánchez et al. For. Pathol. 32:5, 2002. (3) Sánchez et al. For. Pathol. 35:115, 2005.